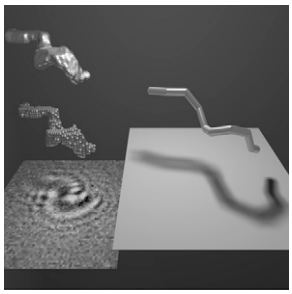
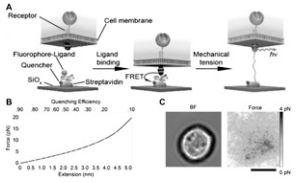
has been studied for some time and there are several competing hypotheses for the underlying mechanics of the axoneme. A key aspect of this work remains unaddressed, however: almost all flagellar waveforms are three-dimensional. Comparing 2D theories with 2D images has met with some success, but for a complete understanding, the 3D nature of the beating must be considered. We have employed digital holographic microscopy to record the 3D beating of a gamete of Plasmodium berghei (a parasite associated with malaria in rodents). When coupled with high-speed imaging we are able to obtain a complete record of the flagellar waveform with excellent temporal and spatial resolution. This sperm is an interesting limiting case: it has no head, and almost no other accessory structures. Our analysis of the 3D beating pattern has implications for the proposed link between structural chirality in the axoneme and the chirality of flagellar waveforms.



1096-Plat
Cell Surface Bound Pericellular Matrix has a Spatially Varying Mesh Size
Louis T. McLane[1], Patrick Chang[1], Anna Granqvist[1], Heike Boehm[2], Anthony Kramer[1], Jan Scrimgeour[1], **Jennifer Curtis**[1].
[1]Georgia Institute of Technology, School of Physics, Atlanta, GA, USA, [2]Max Planck Institute for New Materials and Biosystems, Stuttgart, Germany.
An invisible but thick polymer matrix of polysaccharides extends outward from the surface of many cell types including fibroblasts, smooth muscle cells, and chondrocytes. Grafted to the cell surface, the key backbone polymer hyaluronan is dramatically stretched outward from the surface by large bottlebrush molecules (e.g. aggrecan, versican). This extended, cell bound structure is called the pericellular matrix or cell coat. The shape and thickness of the cell coat is strongly correlated with adhesion-dependent and mechanical activities of the cell in particular proliferation and migration, for example during wound healing, embryogenesis, and cancer metastasis. Yet, much remains to be understood about the cell coat, which is invisible to both phase contrast and DIC microscopy, and which until recently was difficult to fluorescently-label without collapsing its hydrated structure. Our measurements based on optical force probe microscopy and quantitative particle exclusion assays provide some of the first quantitative mechanical and ultra-structural measurements of the cell coat. The assays reveal that cell coat structure on RCJ-P chondrocytes is not crosslinked and further that the pericellular matrix maintains a varying osmotic pressure throughout its structure which influences how objects move through the matrix. We show for the first time that the cell coat has a spatially varying mesh size perpendicular to the cell surface, ranging from approximately ~100nm to 500nm at eight microns. The accessibility of the cell surface through the pericellular matrix both for molecules and nanoparticles as well as the cell's interaction with its surroundings will depend strongly on the cell coat configuration and its varying mesh size (e.g. adhesion to other cells and the extracellular matrix; endocytosis). These measurements provide first experimental clues for how cell coat ultrastructure and mechanics might physically regulate cell interactions with the extracellular environment.

1097-Plat
Fluorescence-Based Tension Probes to Image Force Transmission at Cell Surface
Khalid Salaita.
Emory University, Atlanta, GA, USA.
The interplay between physical inputs and biochemical signaling regulates a significant number of biological processes. A major challenge to elucidating chemo-mechanical coupling lies in the development of new materials and techniques to image forces in cells. Herein, we describe the synthesis and characterization of fluorescence-based turn--on probes for imaging the molecular tension at the surface of living cells in real-time (Nat.Methods 2012). Our sensor takes advantage of FRET between fluorophores as a ''ruler'' and a flexible polymer chain as a reversible spring with a known constant. The linker is comprised of a PEG chain that can be modeled using the worm-like chain model. The sensor allows one to quantify molecular forces with high spatial and temporal resolution for a wide range of receptors and cell types. I will describe the application of these sensors to image forces associated with endocytosis, Notch receptor activation, and integrin adhesion receptors. We measure forces associated with the initial stages of EGF endocytosis (1-4 pN) and show that this force localizes with clathrin light chain. Finally, I will discuss the development of ligation strategies to generate recombinantly expressed protein force sensors to investigate the Notch pathway.



1098-Plat
3 Dimensional Cellular Force Microscopy in Fibrin Gels
Arjun Adhikari[1], Natascha Leijnse[1,2], Leanna Owen[1], Lene Oddershede[2], Alexander R. Dunn[1].
[1]Stanford University, Stanford, CA, USA, [2]Niels Bohr Institute, Copenhagen, Denmark.
The mechanical forces exerted and detected by living cells play integral roles in diverse biological phenomena, including growth and development, wound healing, and cancer metastasis. In the past decade, techniques such as traction force microscopy and micropost arrays have proven to be powerful tools for measuring the forces generated by cells. In particular, traction force microscopy has recently been extended to three-dimensional cell culture environments by embedding tracer beads in either a synthetic polyethylene glycol hydrogel (PEG; Legant et al., Nat. Meth. 2010) or in collagen gels (Koch et al., PLoS ONE 2012). The embedded beads move in response to cell-generated distortions of the matrix, allowing cell-generated forces to be calculated. We sought to develop an experimental system that would exhibit the excellent mechanical properties of the PEG hydrogel while using a naturally occurring biological matrix. Fibrin gels fulfill both of these requirements: fibrin is elastic up to ~50% strain (Brown et al., Science 2009) and is also widely used for 3D cell culture. Here we describe the use of fluorescently labeled fibrin gels to measure the forces generated by cells in 3D culture. We observe dramatic but elastic deformations of the fibrin matrix surrounding cells as they grow, divide, and migrate. Further, we find that the dynamic forces generated by the cell can be measured using the deformations of the matrix itself, providing a direct observation of how the cell modifies its surroundings. We discuss the use of this new technique in studying matrix remodeling and cell migration.

1099-Plat
Magnetic Manipulation of Intracellular Signaling
Fred Etoc[1], Mathieu Coppey[1], Domenik Lisse[2], Yohanns Bellaiche[3], Jacob Piehler[2], maxime dahan[1].
[1]Ecole Normale Supérieure, département de Physique et Biologie, Paris, France, [2]University of Osnabruck, department of Biophysics, Osnabruck, Germany, [3]Institut Curie, Génétique et biologie du développement, Paris, France.
In vivo imaging has shown how the establishment and maintenance of cell polarity relies on complex mechanisms by which signaling cascades become regulated at sub-cellular levels. How signaling networks are spatio-temporally coordinated into a polarized cell is not elucidated. In this context, we developed a new tool to locally probe and perturb signaling pathways inside living cells. In our approach, magnetic nanoparticles (MNPs) functionalized with active proteins are inserted in the cytosol of mammalian cells where they behave as solid signaling platforms. The precise control of MNPs surface chemistry allows to probe the self assembly of macromolecular complexes directly inside the cell. By exerting magnetic forces, MNPs are then manipulated in the cytosol to position their signaling activity at different subcellular locations. The cellular response to this spatially resolved biochemical perturbation is finally quantified in term of effector recruitment and cytoskeleton/membrane dynamics.
We show that MNPs of different sizes, from 50nm to 500nm in diameter, can be used to generate different spatial perturbation patterns. While the biggest MNPs are trapped in internal structures of the cell and require large forces (>10 pN) to be displaced, they allow us to create precise point-like perturbations at the cell periphery. At the opposite, smallest MNPs diffuse fast in the cytosol (~1$\mu m^2/s$) and are used to create gradient of signaling activity spanning over the whole cell.
We apply our technique to the Rho-GTPase signaling network which orchestrate cell polarity and migration. MNPs were functionnalized with GEF or active GTPase. The effect of intracellular GTPase gradient (rac, cdc42) on cell polarity was analyzed. We demonstrated that the pathway linking Rac1 to actin polymerization is spatially restricted to the protrusive areas of the cell by transporting TIAM1 particles at different subcellular locations while monitoring GTPase activation and actin polymerization.

1100-Plat
Lov-Trap: A Broadly Applicable, Genetically Encoded System to Control Protein Activity with Light through Controlled Sequestration at Membranes
Hui Wang[1], Marco Vilela[2], Gaudenz Danuser[2], Andreas Winkler[3], Elisabeth Hartmann[3], Ilme Schlichting[3], Rihe Liu[1], Klaus M. Hahn[1].